CHAPTER

14

Biotechnology for bioenergy production: current status, challenges, and prospects

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14.1 Introduction

Recently, bioenergy has received unprecedented attention from scientists around the world because it has been viewed as a great alternative source of energy to mitigate global warming. With the increasing concerns over climate change, depleting fossil fuel reserves, and the necessity for sustainable energy solutions, bioenergy emerges as a promising alternative to mitigate the adverse impacts of greenhouse gas emissions associated with fossil fuel combustion (Al-Ahmad, 2018; Zabermawi, Alsulaimany, El-Saadony, & El-Tarabily, 2022; Zhu, Abdelaziz, Hulteberg, & Riisager, 2020). As the global population continues to grow, there is an increasing demand for renewable and sustainable energy sources. Bioenergy is a type of energy generated from biomass (biodegradable part of products, residues, and wastes) obtained from plant and animal origin (Fig. 14.1). A wide variety of biomass sources

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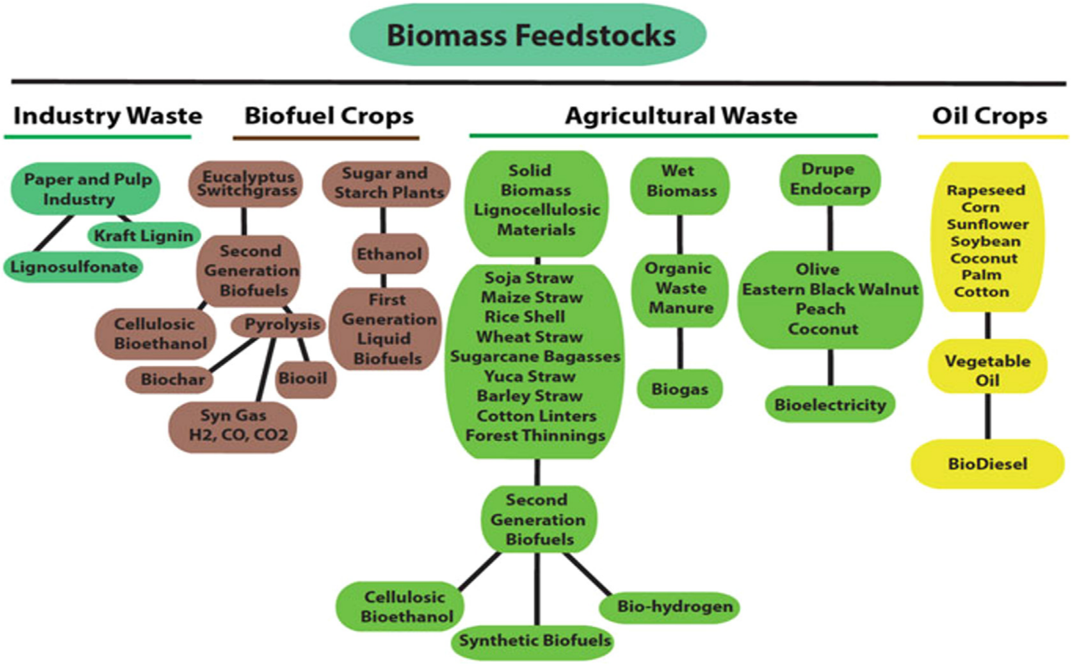

FIGURE 14.1 Biomass feedstocks and their utilization in the production of biofuels, bioenergy, and bioproducts (Welker et al., 2015). Source: *Adapted from Welker, C., Balasubramanian, V., Petti, C., Rai, K., DeBolt, S., & Mendu, V. (2015). Engineering plant biomass lignin content and composition for biofuels and bioproducts. Energies, 8, 7654–7676.*

such as food, fibers, industrial, agricultural, and municipal wastes, residues from forest, and so forth can be used to generate different forms of bioenergy (electricity and combined heat and power).

Bioenergy involves utilizing renewable biological resources, such as plants, crops, agricultural residues, and certain types of waste collectively known as biomass, to generate heat, electricity, or biofuels (Fig. 14.2) (Calvin et al., 2021). Different researchers have employed various agricultural wastes in bioethanol production. These include barley straw (Serrano et al., 2018), coffee husk (Gouvea, Torres, Franca, Oliveira, & Oliveira, 2009), grasses (Scordia, Testa, & Cosentino, 2014), corn stover (Dhiman, David, Braband, Hussein, & Sani, 2017), groundnut shell (Bhatt, 2014), pine (Vaid, Nargotra, & Bajaj, 2018), sawdust (Lynd, Weimer, Van Zyl, & Pretorius, 2002), switchgrass (Xu, Singh, & Himmel, 2009), waste paper (Nishimura et al., 2017), spruce (Crawford et al., 2016; Mirahmadi, Kabir, Jeihanipour, Karimi, & Taherzadeh, 2010), wheat straw (Wi, Choi, Kim, Kim, & Bae, 2013), and water hyacinth (Kumar, Barrett, Delwiche, & Stroeve, 2009).

Generally, biomass-derived fuels are broadly known as biofuels, which may be in solid, liquid, or gaseous state. Examples include bio-oil, ethanol, biodiesel, Fischer–Tropsch (FT) hydrogen, methanol, and methane (Bahadar & Khan, 2013; Joshi, Pandey, Rana, & Rawat, 2017; Kour et al., 2019). Biofuels offer diverse benefits like being renewable, release of fewer toxic compounds during combustion, and no emission of CO_2 into the atmosphere

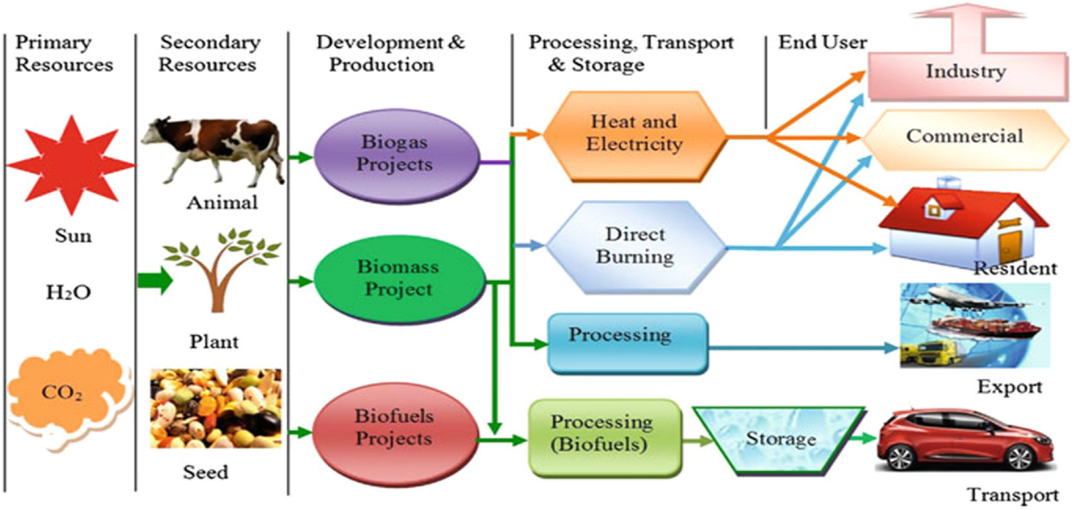

FIGURE 14.2 Biofuel supply chain from primary resources to the end user (Azad et al., 2015). Source: *Adapted from Azad, A. K. Rasul, M., Khan, M. M. K., Sharma, S. C. & Hazrat, M. (2015). Prospect of biofuels as an alternative transport fuel in Australia. Renewable and Sustainable Energy Reviews, 43, 331–351.*

since organisms producing biomass absorb greater part of the CO_2 released (Al-Ahmad, 2018; Kour et al., 2019; Razzak, Hossain, Lucky, Bassi, & de Lasa, 2013; Surriya, Saleem, Waqar, Kazi, & Öztürk, 2015; Voloshin, Rodionova, Zharmukhamedov, Veziroglu, & Allakhverdiev, 2016). Biomass still remains one of the world's largest sustainable energy resources with various forms of energy sources, which have been considered a great substitute to conventional fossil fuels. Scientists have proposed that by the year 2050, biomass and consequently bioenergy will contribute between 15% and 50% of the world's total energy consumption (Kour et al., 2019; Kumar et al., 2010).

Among the bioenergy sectors, transport biofuels are experiencing significant growth, with production projected to increase by 6%–8% annually (Bauen, Berndes, Junginger, Londo, & Vuille, 2009). The production of first-generation biofuels, such as bioethanol derived from starch and sugar crops, and biodiesel obtained from oil crops and residual fats, has faced social and environmental challenges. These challenges include the potential impact on food crop prices and interference with arable land use. To address these concerns, recent research has focused on ensuring that bioenergy crops do not compete with food production or interfere with arable land utilization (Zabermawi et al., 2022).

Biotechnology is a promising technology that has revolutionized the field of bioenergy production, enabling significant advancement in the yield, quality, and sustainability of bioenergy products. Biotechnological approaches have been employed for the production of second-generation biofuels and other energy products. These approaches rely on non-food biomass sources, such as lignocellulosic feedstocks, including organic wastes, forestry residues, high-yield woody or grass energy crops, and algae. By utilizing non-food biomass, the pressure on food crops and land use can be alleviated (Sakuragi, Kouichi, & Mitsuyoshi, 2011; Zabermawi et al., 2022; Zhu et al., 2020). Modern biotechnological techniques, such as genetic engineering, are utilized to modify the genetic characteristics of energy-dedicated plants, including forest woody species. This optimization aims to enhance traits such as high cellulose and lignin content, increased biomass yield, and resistance to pests and diseases (Rodionova et al., 2017).

Furthermore, biotechnological approaches play a vital role in developing more efficient and cost-effective techniques for converting biomass into bioenergy (Adegboye, Ojuederie, Talia, & Babalola, 2021). The process of breaking down the intricate cell walls of lignocellulosic plant materials into simple sugars is complex and expensive (Binod, Gnansounou, Sindhu, & Pandey, 2019); however, through biotechnology, particularly synthetic biology, it becomes possible to engineer biological systems for novel purposes. This leads to the creation of more efficient enzymes and robust microbes. Synthetic biology enables the production of novel enzymes that accelerate biomass breakdown and the modification of microorganisms to directly generate bioenergy (Adegboye et al., 2021; Binod et al., 2019; Zabermawi et al., 2022).

In light of the significant role of biotechnology in bioenergy, this chapter aims to provide a comprehensive overview of the past, present, and future of biotechnology in bioenergy production. It will delve into the historical milestones, current state-of-the-art technologies, and emerging trends in the field. This chapter will also explore the various biotechnological approaches employed, including microbial engineering, enzyme engineering, algal biotechnology, and metabolic engineering. Additionally, it will examine the challenges and opportunities as well as the future perspectives on the integration of biotechnology with other renewable energy sources.

14.2 Historical overview of biotechnology applications in bioenergy

Early biotechnology applications in bioenergy were geared toward selective breeding of hydrocarbon yielding plants such as Euphorbia that are capable of producing bioenergy products. Once suitable species were screened and identified, in vitro micropropagation was carried out for mass production (Agarwal & Kumar, 2018). Traditional conversion methods of biomass involved the use of thermal or chemical methods, which are rather intensive and further contribute to environmental pollution. With the advent of biotechnology, new solutions are created to address these challenges (Amoah, Kahar, Ogino, & Kondo, 2019). As the demand for bioenergy increased, biotechnology offered opportunities to produce various types of alcohols through the fermentation of sugars derived from biomass hydrolysis. These alcohols find applications in diverse industries, including biofuels, pharmaceuticals, cosmetics, and more.

During this time, optimizing the fermentation process of sugars obtained from the hydrolysis of starch and sugar crops became a necessity, leading to the development and utilization of microorganisms capable of efficiently converting sugars into desirable bioproducts, such as ethanol. Various strains of yeast and bacteria were explored to enhance fermentation efficiency and yield. *Saccharomyces cerevisiae*, which can grow on both simple sugars and glucose, were the most employed and most used in the fermentation process. *Zymomonas mobilis*a Gram-negative bacterium, which has the ability to produce more ethanol yield at a faster rate than *Saccharomyces*, was also often employed; however, it is not well suited for all of the biomass resources conversion because it ferments only glucose, fructose, and sucrose.

With advancement in knowledge, metabolic engineering was then introduced to manipulate these organisms and enhance their capabilities to get more output. Metabolic engineering allowed for the development of nonnative organisms for the production of compounds beyond the scope of what native organisms can produce. For example, n-butanol fermentation in *Clostridium* has been used since the early 1910s. However, the relatively slow growth rate and spore-forming life cycle of *Clostridium* limited its use in industrial fermentation. Furthermore, the relatively unknown genetic system and complex physiology of the microorganism present difficulties in engineering its metabolism. To address this issue and for increased butanol production, the genes responsible for n-butanol production in *Clostridium acetobutylicum* were successfully cloned and expressed in *E. coli* using expression plasmid (Atsumi & Liao, 2008). Woodruff, May, Warner, and Gill (2013) developed a strain of *E. coli* engineered for ethanol production. The genes responsible for homoethanol production were integrated into the genome of the bacterium using an inducible promoter, and the genes encoding four enzymes from competing pathways were deleted. The strain was capable of producing more than 30 g/L of ethanol from any byproduct as compared to native organisms.

As the need for bioenergy products continued to increase, starch crops were genetically modified to enhance biomass production. By targeting genes that control factors such as cell division, cell expansion, and carbon allocation, researchers were able to enhance the overall biomass production in various starch crops. However, the utilization of food crops for bioenergy production soon posed a significant challenge as it resulted in the escalation of food prices. Consequently, researchers began exploring alternative sources of biomass for biofuel production, such as nonedible agricultural residues, industrial byproducts, and organic wastes from municipalities collectively known as lignocellulosic biomass. However, utilizing lignocellulosic biomass for bioenergy production presented its own set of challenges. The intricate composition of lignocellulosic materials and their high lignin content made it difficult to break them down into sugars through hydrolysis. Nonetheless, recent advancements in biotechnology have offered viable solutions to address this obstacle.

Bioenergy production is classified into four generations based on the biotechnological developments and biomass used (Kricka, Fitzpatrick, & Bond, 2014). The first-generation bioenergies (i.e., bioiesel and bioethanol) are produced from edible crops such as maize, oil seeds, and sugarcane and compete with the agricultural/food sector, which can eventually result in food shortage for the future generations. The advantages of first-generation bioenergies include their ecofriendliness, biodegradability, and provision of high quality energy, while their limitations include their high cost, endangering food security through the usage of edible crops, among others, as listed in Table 14.1.

These limitations (as listed in Table 14.1) spurred scientists' interest in developing the second-generation bioenergy, which employs nonedible feedstocks, especially lignocellulosic biomass such as residual and waste products from household and industries. This was seen as a feasible alternative to edible crops, as it does not affect the availability of food. The major limitation of the second-generation bioenergy is the need for intensive energy pretreatments saccharification and hydrolysis. In addition, lignocellulosic biomass is highly recalcitrant and requires high-cost investment and intensive labor for processing (Wheals, Basso, Alves, &

TABLE 14.1 Advantages and limitations of first-generation bioenergy.

Advantages	Limitations
Process is ecofriendly and biodegrable	It is expensive It may result in the loss of biodiversity due to monoculturing practices
Provide high-quality energy Less contribution to the greenhouse effect Source of employment	Irrigation of bioenergy plants may be cumbersome
Contributes greatly to sustainable development Reduces high dependency on fossil fuels Less pollution of the environment	It enhances the use of genetically modified plants It endangers food security with the use of edible crops in first-generation bioenergy

Amorim, 1999). The third- and fourth-generation bioenergy was developed by algal biomass and improved algal strains (a product of metabolic engineering) (Meneses, Raud, Orupold, & Kikas, 2017). These two generations are being considered as the major and significant source of biodiesel in the future (Littlejohns, Rehmann, Murdy, Oo, & Neill, 2018).

14.3 Biological systems and biotechnological approaches for bioenergy production

Bioenergy is produced using different biological resources through several techniques and technologies. In past few decades, the prominent recognized sources of bioenergy are biomass of plants and microorganisms, which have been described as being ecofriendly (Dragone, Fernandes, Vicente, & Teixeira, 2010; Heimann, 2016). Due to the ability of plants and algae to photosynthesize, they are differentiated from other bioresources for biomass accumulation through the process of formation of sugar using atmospheric carbon dioxide in the presence of sunlight (Voloshin et al., 2015). Bioenergies are used in developing countries as an energy source (Dragone et al., 2010; Koh & Ghazoul, 2008; Röder & Welfle, 2019). In the United States, for the past decades, the consumption of bioenergy accounts for approximately 50% of the total renewable energy forms (Christopher, Kumar, & Zambare, 2014). The top producers of bioenergy have identified a lack of good energy policies and nonavailability of biomass and efficient technology as the major factors affecting the production and utilization of bioenergy (Amoah et al., 2019). Bioenergy production involves the conversion of the chemicals stored up in biomass into usable forms that can be used to generate energy. Previously, this is greatly achieved through different traditional methods (physical/thermal or chemical), which is known to be laborious and also not ecofriendly (Baeyens et al., 2015; Christopher et al., 2014); however, with the use of the biotechnological approach, these challenges have been substantially addressed.

14.3.1 Bioethanol production

14.3.1.1 Biological resources for bioethanol production

Bioethanol, a liquid biofuel, is produced through different conversion technologies using biomass feedstocks with the help of microbes. Bioethanol has been recognized worldwide for providing energy, with a continually increasing production demand. Bioethanol is still the largest supplier of bioenergy with the total global shares of the United States and Brazil standing at 85%−90% (Baeyens et al., 2015). In order to avoid competition with food crops, which eventually may lead to food crises, the use of nonedible crops (like lignocellulosic biomass) has been recommended to be a feasible and sustainable alternative (Baeyens et al., 2015; Balat, Balat, & Öz, 2008). Its production process takes place in different stages, including preparation of biomass feedstock, pretreatment, hydrolysis, or saccharification, using bacterial and fungal enzymes, followed by fermentation using diverse microbes (bacteria, yeast, and fungi), and ends with distillation and dehydration process (Fig. 14.4). The choice of the pretreatment strategy employed determined to a large extent the yield of bioethanol produced using different substrates. Many researchers have reported production of bioethanol utilizing different microbes in a five-step process (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010; Amoah et al., 2019; Binod et al., 2010; Ho et al., 2013; Kim & Dale, 2004).

14.3.1.2 Fermentation technology for bioethanol production

Fermentation is the key component where advancement in technology plays a key role and is required to be feasible. The fermentation process of lignocellulosic biomass in bioethanol production employs mainly bacteria and yeast to convert sugars to ethanol. For the maximum yield, the organism chosen should be able to withstand lower pH and higher temperatures, form minimal byproducts, and should possess tolerant characters toward inhibitors, sugars, and ethanol concentrations (Jahnavi, Prashanthi, Sravanthi, & Rao, 2017). Different fermentation technologies are employed in bioethanol production, the most common ones being simultaneous saccharification and fermentation (SSF), separate hydrolysis and fermentation (SHF), consolidated bioprocessing (CBP), and simultaneous saccharification and cofermentation.

The SSF requires minimal equipment, low capital investment, a simplified operation, and no inhibition of end products by glucose; thus, it increases the saccharification rate and ethanol yield. However, the process consumes more time and produces a reduced yield of fermentable sugar, which subsequently results in a lower yield of ethanol. The CBP requires the use of a small number of vessels for fermentation, involves reduced capital cost and a simplified operation with minimal risk of contamination by glucose levels, and product inhibition by cellulose (Hasunuma & Kondo, 2012). The major disadvantage of the CBP is the lack of thermophilic microorganisms suitable for the process. For SHF, both hydrolysis and fermentation processes are carried out separately at optimum temperatures. As a result, the process is time-consuming and also costly. The SSCF process minimizes the use of reactors, reduces capital investment cost, and results in high productivity of bioethanol. The major limitations are that it involves loading of high enzymes and it operates at different optimum temperatures for hydrolysis and the fermentation microbes (Choudhary, Surender, & Lata, 2016).

14.3.2 Biodiesel production

14.3.2.1 Biological resources and technology for biodiesel production

Different researches have established biomass as a major source for renewable energy (Berndes, Hoogwijk, & Van den Broek, 2003; Demirbas, 2005; Lund, 2007). Biodiesel can be produced from oils (such as animal oil/fats, vegetable oil, or waste cooking oil). These oils are converted by a process known as transesterification. This process involves three basic steps: base-catalyzed transesterification, direct acid-catalyzed transesterification of the oil, and finally the conversion of the oil to its fatty acids and subsequently to biodiesel. Some plants known to contain oil have been used extensively in biodiesel production. For instance, soybean (which contains five different fatty acids—linolenic, linoleic, palmitic, stearic, and oleic), palm fruits, sunflower, and *Jatropha* seeds have all been employed to produce biodiesel (Kour et al., 2019). However, a study by Phan and Phan (2008) revealed that biodiesel produced using vegetable oil could be 50% more expensive than those produced from waste cooking oils. Apart from these oils, microorganisms are also a good source for biodiesel production. Different microbes including bacteria, cyanobacteria, algae, and yeasts such as *Rhodosporidium toruloides*, *Trichosporon pullulan*, *Lipomyces lipofer*, *Cryptococcus albidus*, and *Lipomyces starkeyi* have the potential to produce lipids, which can be used for biodiesel production (Fei, Chang, Shang, Kim, & Kang, 2011; Fu, Fei, Shang, Brigham, & Chang, 2018; Meng et al., 2009).

14.3.3 Biogas production

Generally, biogas production occurs in four steps—hydrolysis, acidogenesis, acetogenesis, and methanogenesis. For instance, in biomethane production, the hydrolysis procedure depends on the structure of the substance employed as a substrate (such as proteins, carbohydrates, lipids, and lignocelluloses) (Kour et al., 2019). In this process, some fermenting bacteria like *Bifidobacteria*, *Clostridia*, and *Bacteriocides* convert complex biopolymeric molecules (proteins, carbohydrate, and lipids) into simpler organic molecules (amino acids, sugar, and fatty acids). The other processes of acidogenesis and acetogenesis involve the production of soluble molecules such as biohydrogen, carbon dioxide, and acetate using different bacteria, while methanogenesis produced biomethane and carbon dioxide using methanogens (strict anaerobic archaea).

14.3.3.1 Biological resources and technology for biogas production

14.3.3.1.1 Biohydrogen production

Biohydrogen (a biogas) is produced through diverse biological routes. To obtain the maximum yield from a substrate, the choice of the route of production is of great priority. Biohydrogen has been produced extensively by employing different fermentative microbes such as *Bacillus*, *Clostridium*, and *Enterobacter* (Fabiano & Perego, 2002; Kotay & Das, 2007). This process occurs under anaerobic conditions, with H_2 produced as a byproduct of the oxidative catalytic process of organic substrates using the endogenous hydrogenase catalyst produced by the microorganisms. On the other hand, methane is produced by a group of microbes (methanogens) such as *Methanosaeta concilii*, *Metanonococcus mazei*, and *Methanosarcina barkeri*. Some members of the methanogenic genera are able to utilize organic acids (e.g., as acetic acid) as a substrate for methane production (Zhang et al., 2019). Biogas production is of particular interest because of the fundamental role it can play in waste treatment and management.

14.3.3.1.2 Bimethanol production

Suntana, Vogt, Turnblom, and Upadhye (2009) described biomethanol as the most effective biofuel for the generation of power. Biomethanol has a wide variety of applications such as in fuel cell-powered vehicles and liquid hydrogen carriers serving as a hydrogen storage compound. Also, its physical and chemical characteristics make it more attractive. In addition, biomethanol is placed ahead of gasoline, as it burns at a very low temperature (Shamsul, Kamarudin, Rahman, & Kofli, 2014). For the maximum economic and environmental benefits, employing lignocellulosic wastes for methanol production is considered most favorable (Chandra, Takeuchi, & Hasegawa, 2012). Different agricultural residues have been used successfully in biomethanol production. These include rice husk, bran, straw, banana peel, and other plant biomass (Anitha, Kamarudin, Shamsul, & Kofli, 2015; Arteaga-Pérez, Gómez-Cápiro, Karelovic, & Jiménez, 2016). Till now, production of biomethanol from agrowastes has not been fully exploited; additional investigations are required for utilization of these wastes for its production.

Biogas production from municipal wastes is significantly predisposed by the type of microbial communities present. For the potential conversion of wastes to biogas, an analytical study revealed three main classes of organic wastes—municipal, agricultural, and industrial wastes. A study by Melikoglu, Lin, and Webb (2013) projected that agricultural waste will increase with the increasing human populations. This waste made up largely of the remains of crops and animal manure is known to contain large amounts of carbohydrates that can be converted to sugars, which by fermentation can subsequently produce biogas. Similarly, some industrial wastes also contain fermentable sugars, which can serve as substrates for hydrogen fermentation. Various bioresources have been successfully employed in biogas production. Cheese whey from the cheese industry has been identified to produce high yield of hydrogen gas (Azbar, Dokgöz, & Peker, 2009; Davila-Vazquez, Alatriste-Mondragón, de León-Rodríguez, & Razo-Flores, 2008), noncomparable with other biomass sources. Other potential biomass sources for biogas production include some industrial wastes such as rice slurry, cassava waste, and oil seed mill effluent production (Kemausuor, Addo, & Darkwah, 2015; Mohammadi, Ibrahim, Annuar, & Law, 2011; Mussoline, Esposito, Giordano, & Lens, 2013).

14.4 Biotechnological approaches for bioenergy production

The growing energy demands driven by population growth, along with the urgent need to address climate change and the depletion of fossil fuel reserves, have necessitated the adoption of bioenergy solutions. This has led to the adoption of advanced biotechnological approaches to effectively utilize lignocellulosic biomass and meet the rising demand for bioenergy. Some of the modern biotechnological approaches that are used today are discussed below.

14.4.1 Enzyme engineering

Lignocellulosic biomass has a recalcitrant nature and requires degradation to breakdown their complex structure. However, to breakdown the complex structure into fermentable sugars, a high amount of commercial enzymes is needed, which contributes to the cost of production (Amoah et al., 2019; Gonçalves et al., 2015). This has been a major bottleneck in the use of lignocellulosic biomass for the production of bioenergy products. To address this, several biotechnological techniques, which involve enzyme manipulation, have been explored to achieve an optimized amount of sugar with reduced use of enzymes. It is worthy to note that different feedstocks subjected to different pretreatments contain different (and variable) concentrations of different sugars and hence require different enzyme combinations for their hydrolysis (Banerjee, Scott-Craig, & Walton, 2010). Enzymes are engineered through immobilization, where an enzyme is attached to a solid support, which enhances its stability, and through protein engineering, where the structure of the enzyme is completely altered to improve its performance (Ali, Ishqi, & Husain, 2020).

Several studies have reported the usefulness of cellulase supplemented with accessory enzymes like xylanases, acetyl xylanesterases, and feruloyl esterases in hydrolyzing the complex intermolecular bonds, which exist within hemicellulose, as well as interconnecting linkages of lignin and hemicellulose, therefore reducing the amount of enzyme used. Because of the synergy that exists between xylase and cellulase and because of the high amount of xylan in the plant cell wall, xylases are the most widely used accessory enzyme in the hydrolysis of hemicellulose. Gonçalves et al. (2015) reported a twofold increase in the resulting sugars and an increased hydrolytic activity of cellulase when a combination of xylanase from glycoside hydrolase family 10 (GH10) and glycoside hydrolase family 11 (GH11) was used during the hydrolysis of a pretreated bagasse, indicating the synergistic effect between these enzymes. Efforts are ongoing to develop thermostable enzymes capable of efficiently hydrolyzing this complex plant material with minimal sugar loss.

14.4.2 Metabolic engineering

Metabolic engineering aims to improve the production of economically valuable molecules through the genetic manipulation of microbial metabolism (Volk et al., 2022). Because of the recalcitrant nature of lignocellulosic biomass due to the high lignin content in their cell wall, this technology enables the modification of metabolic pathways of organisms for increased bioproduct production. One way through which the technology is used is in the production of alternative lignin. This involves manipulating the metabolic pathways within plants to redirect the flow of metabolites toward the production of the desired alternative monomers. This can be achieved through the cloning of biosynthetic pathways and the mutation of endogenous genes to enhance the production of specific monomers. The resulting engineered lignins are designed to be more easily hydrolyzed and have reduced recalcitrance, facilitating the breakdown of biomass into fermentable sugars and enabling efficient biomass conversion (Mottiar, Vanholme, Boerjan, Ralph, & Mansfield, 2016; Simmons, Loqué, & Ralph, 2010; Vanholme et al., 2012).

Another approach in metabolic engineering is the development of microorganisms that can efficiently convert carbohydrates from biomass into useful bioproducts. Once hydrolyzed from biomass through various pretreatments, polysaccharides pass through metabolic pathways in microbial cell factories. For example, glucose typically passes through the glycolysis pathway to produce intermediates such as Acetyl-CoA and pyruvate, which are used as building blocks for the production of bioenergy products like alcohols. Traditional metabolic pathways have limitations, such as carbon loss, complexity, and low product yield. Metabolic engineering has been employed to create strains with novel metabolic pathways, leading to improved yields from minimal feedstock (Kim, Hwang, & Lee, 2022). In the work presented by Liang, Chen, Liu, and Wen (2018), the Embden Meyerhof pathway in *E. coli* was replaced with a heterologous Entner−Doudoroff pathway adapted from *Zymomonas mobilis* for isobutanol production from glucose fermentation. This led to over 50% increase in isobutanol production. Similarly, introducing the phosphoketolase pathway into *Saccharomyces cerevisiae* led to increased polyhydroxybutyrate production from glucose (Kocharin, Siewers, & Nielsen, 2013). A nonphosphorylating pathway (Dahms pathway) was introduced into *E. coli* to produce glycol (Cabulong et al., 2017) and glycolic acid (Cabulong, Bañares, Nisola, Lee, & Chung, 2021) from xylose, and the maximum yield was obtained.

Recent studies have focused on developing consortia of strains capable of simultaneously fermenting glucose and xylose (Kim et al., 2022). Metabolic engineering can also be applied to harness carbon dioxide (CO_2) as a feedstock for bioenergy production. By genetically manipulating microorganisms, it is possible to redirect carbon flux toward the synthesis of value-added compounds, such as biofuels and biochemicals, using CO_2 as a carbon source. This approach not only contributes to CO_2 mitigation but also provides a sustainable route for utilizing carbon emissions (Volk et al., 2022).

14.4.3 Genetic engineering of energy crops

To overcome the recalcitrance nature of the cell wall of energy crops, genetic engineering has been used to modify the properties of these crops to enhance cellulose synthesis, enhance plant growth and cell wall porosity and solubility, and increase the sugar yield, following enzymatic hydrolysis. These approaches are directed toward modifying the lignin content, reducing cell wall crystallinity and altering the hemicellulose−lignin complexes (Abramson, Shoseyov, Hirsch, & Shani, 2012). There are two main approaches to reduce the amount of lignin in plants: by decreasing the expression of genes responsible for producing monolignols, which are the building blocks of lignin, or by reducing the activity of genes that encode the enzymes involved in the process of lignin polymerization. These methods aim to limit the availability of monolignols or hinder the polymerization of lignin, ultimately leading to a reduction in lignin content (Vermerris & Abril, 2015).

Genetic engineering and enzyme technologies have been successfully employed in strain improvement of various microbes in the past few decades to enhance ethanol production (Cavalheiro & Monteiro, 2013; Divate, Chen, Divate, Ou, & Chung, 2017; Koppolu & Vasigala, 2016; Selim, El-Ghwas, Easa, & Hassan, 2018). The production of bioenergies from renewable biomass has been initiated in several countries, including America, Brazil, China, France, India, Germany, and Thailand (Gnansounou, 2010; Swart, Ho, & Jiang, 2008). Among these countries, the world frontier leaders of bioethanol production remain the United States and Brazil, with a production capacity of about 60% of the world's biofuel production. The study by Van Acker et al. (2013) investigated the impact of genetic mutations in monolignol biosynthetic genes on lignin content and composition in

Arabidopsis plants. They found that lignin content plays a crucial role in biomass conversion efficiency, as it affects the yield of sugars obtained during saccharification. Specifically, Berthet et al. (2011) found that mutations in the genes LAC4 and LAC17, which encode laccase enzymes involved in lignification, resulted in a 20% reduction in lignin content. The double mutant (lac4/lac17) exhibited a 40% decrease in lignin content without affecting plant growth. Furthermore, the double mutant showed a threefold increase in glucose yield during biomass conversion, even without pretreatment (Berthet et al., 2011). These findings highlight the potential of reducing lignin content through genetic modifications to enhance biomass conversion efficiency.

Recent research has focused on genetically engineering plants to changing the composition of sugars in plant cell walls. The reason behind this is that C6 sugars are more efficiently converted into bioproducts like ethanol compared to C5 sugars. It is possible to modify plant cell walls to have less of a xylan or to add more galactan or another mixed-linkage sugar, glucan. These modifications result in an overall increase in C6 sugars. Importantly, these changes in sugar composition did not negatively affect plant growth (Brandon & Scheller, 2020; Shih, Liang, & Loque, 2016). This is achieved by overexpressing certain genes responsible for production of C6 sugars; however, careful consideration of promoters may be critical for the success of overexpressing these genes, as certain studies have recorded reduced cellulose content from overexpressing certain genes (Mazarei et al., 2018; Tan et al., 2015).

14.4.3.1 Recombinant DNA technology

Recently, the application of genetic engineering and recombinant DNA technology in bioenergy production is gaining more attention. This technology is used to improve the strains of industrially significant microorganisms in order to meet the demand of sustainable and alternative energy necessitated by the limitations of fossil fuels. Also, to overcome inhibitory conditions, recombinant DNA technology is a very useful approach for manipulation and regulation of stress-tolerant genes (Lugani, Sooch, & Kumar, 2019). This technology offers great benefits such as increased rate-determining step and provides an extensive pathway for product production and enzyme engineering for the production of the desired products (Dogan, Demirci, Aytekin, & Sahin, 2014). Different researchers have reported the use of genetically engineered recombinant strains of microorganisms in improving production of bioethanol (Dogan et al., 2014; Ge et al., 2014; Ko et al., 2018; Kricka et al., 2014; Sar, Stark, & Akbas, 2017). Deswal, Gupta, Nandal, and Kuhad (2014) reported that genetically modified strains of fungi were efficiently used to produce fermentable sugars from agricultural biomass of corn stover, sugarcane bagasse, straw, and so forth.

Ge et al. (2014) synthesized three recombinant strains of *Saccharomyces cerevisiae*, HDY-ZMYWBG1, HDY-ZMYWBG2, and ZMYWBG3, for bioethanol production through lithium acetate transformation. Of these strains, HDY-ZMYWBG1 produced the maximum yield of ethanol.

Currently, recombinant DNA technology is one of the techniques used for genetic manipulation of microorganisms to improve saccharification and fermentation processes. Others include selection, adaptation, mutation, and protoplast fusion. While both pentose and hexose sugars can be utilized for ethanol production by a number of species of yeasts, like *C. shehatae, P. stipitis*, and *P. tannophilus, S. cerevisiae* can only utilize hexose sugars for production ethanol (Lin & Tanaka, 2006). Also, Hasunuma and Kondo (2012) applied cell surface engineering to develop recombinant thermophilic *Kluyveromyces marxianus* strain for ethanol production. Similarly, recombinant strains of *S. cerevisiae* and *E. coli* ZSC113 employed in batch culture fermentation result in improved ethanol production (Parambil & Sarkar, 2015). Ko et al. (2018) also reported the production of ethanol from lignocellulosic wastes using engineered feedstock and microbes.

14.4.4 Algae biotechnology

The demand for bioenergy to replace fossil fuels is on the increase owing to the fact that the amount of fossil fuels currently produced is unable to meet the requited amount consumed. The potential usage of algae as a biofuel feedstock has received great attention during the last decade since this route of production efficiently reduces CO_2 emissions (Merlo, Gabarrell Durany, Pedroso Tonon, & Rossi, 2021; Mondal et al., 2017), as a result conserving and maintaining good balance of the environment compared to fossil fuels. Bioenergy produced from algae has been shown to prevent global warming by sufficiently reducing CO_2 emissions by a considerable 78.5% compared to petroleum-based energy (Van Gerpen, 2005). Algal biofuels offer great benefits: (1) they are extremely ecofriendly, that is, nontoxic to the environment; (2) they are cheap, that is, readily available source; (3) they have long-term sustainability and biodegradable effect; and (4) they contribute efficiently to the carbon cycle when burned (Ahmad, Buang, & Bhat, 2016). Fig. 14.3 shows a general outline of the different pretreatment and fermentation methods involved in the synthesis of bioethanol from microalgae.

Algae have been investigated as an alternative source of bioenergy, which is free from the setbacks of first- and second-generation bioproducts due to their rapid growth rate and ability to survive in extreme conditions (Chia et al., 2018). Algae are capable of storing carbohydrates as energy reserves in forms such as starch, cellulose, xylose, and galactose

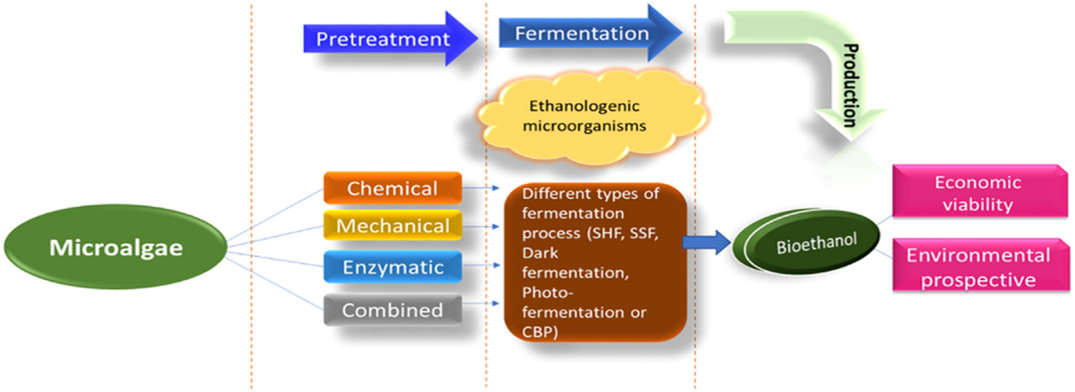

FIGURE 14.3 Adapted: A general general outline of the different pretreatment and fermentation methods involved in the synthesis of bioethanol from microalgae (Phwan et al., 2018). Source: *Extracted from Phwan, C.K., Ong, H.C., Chen, W.-H., Ling, T.C., Ng, E.P., Show, P.L., 2018. Overview: comparison of pretreatment technologies and fermentation processes of bioethanol from microalgae. Energy Conversion and Management, 173, 81–94.*

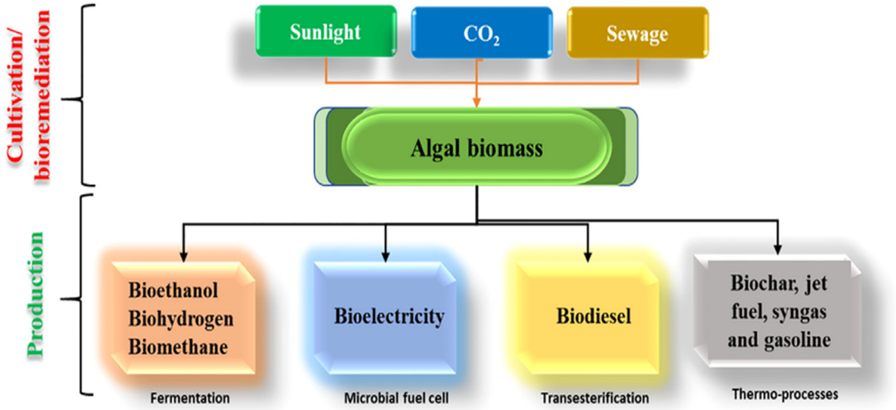

FIGURE 14.4 Adapted: Production of various biofuels from algae biomass (Saad et al., 2019). Source: *Extracted from Saad, M.G., Dosoky, N.S., Zoromba, M.S., & Shafik, H.M. (2019). Algal biofuels: current status and key challenges. Energies, 12, 1920.*

with low lignin content (Chen et al., 2013; Chia et al., 2018). They have been found to have similar properties with lignocellulosic biomass (Yun, Smith, deNoyelles, Roberts, & Stagg-Williams, 2014). The hydrolysis of this algae release sugars like glucose and other monomers depending on the algae type. These sugars are further fermented to release useful bioproducts (Ho et al., 2013). The different processes in the conversion of algal biomass to different bioenergies/biofuels are shown in Fig. 14.4.

The most important biofuels derived from algae include bioethanol, biodiesel, biogas, and biohydrogen (Hussain et al., 2021). Microalgae such as *Saccharomyces cerevisiae*, *Chlorella* sp., *Botryococcus* sp., *Scenedesmus* sp., and *Picochlorum* sp. are known to have lots of oleic acid embedded in them, and this has been reported to improve the oxidative stability of biodiesel (Milano et al., 2016). Also, a number of algal species have been identified as potential bioethanol producers. These include *Spirogyra* sp., *Sargassum* sp., *Prymnesium parvum*, *Gracilaria* sp., *C. sorokiniana*, and *Laminaria* sp. (Behera et al., 2015; Constantino, Rodrigues, Leon, Barros, & Raposo, 2021; Rajkumar, Yaakob, & Takriff, 2014). Microalgae are efficient sources of bioenergy, as they are very easy to harvest, grow faster compared to lignocellulosic biomass, have an easy conversion rate, are easy to cultivate, require small land, and contain high amounts of lipids and polysaccharides (Ahmad, Banat, Alsafar, & Hasan, 2022).

14.5 Conclusion and future perspectives

This chapter discussed the past, present, and future of biotechnology in bioenergy. The recent remarkable advancement recorded in the research of biomass-generating energy is due to serious challenges of depletion of oil reserves, energy security, environmental degradation, and climate changes the modern world is facing. In this regard, bioenergy is expected to mitigate these limitations in a very sustainable and ecofriendly manner. The application of biotechnology in bioenergy production as discussed in this chapter has helped increase and optimize the quality. There is no doubt that bioenergy has become a rapidly growing research field, and significant successes have been recorded in the application of biotechnology for its production. However, different integrated technologies of engineering and biology are still necessary for optimizing bioenergy production at the commercial level. Therefore, the combination of multiple genetic engineering technologies is considered the best for optimizing and obtaining the desired bioenergy. In addition, in order to achieve a sustainable bioenergy economy in the future, there is a great need to fully understand the effect of climatic changes on the production of bioenergy.

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Further reading

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